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Short Communication

Residual HIV-RNA Levels Persist for Up to 2.5 Years in Peripheral Blood Mononuclear Cells of Patients on Potent Antiretroviral Therapy

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ABSTRACT

The long-term response of 10 asymptomatic, antiretroviral therapy-naïve HIV-1-infected patients to potent combination antiretroviral therapy was characterized by monitoring levels of HIV-1 RNA in plasma, peripheral blood mononuclear cells (PBMC), and lymphoid tissue using highly sensitive HIV-1 RNA assays. Although plasma viral loads were continuously suppressed to levels below 50 HIV-1 RNA copies/ml for up to 2.5 years (60–128 weeks), HIV-1 RNA was still detectable at very low levels (1 to 49 HIV-1 RNA copies/ml) in 25% of the samples. In corresponding PBMC specimens, residual HIV-RNA was detectable in as much as 91% of samples tested (1 to 420 HIV-1 RNA copies/ μ g total RNA). Similarly, HIV-1 RNA levels in lymphoid tissue also remained detectable at a high frequency (86%). A highly significant correlation was demonstrated between therapy-induced change in PBMC HIV-1 RNA levels and change in plasma HIV-1 RNA levels ($r^2 = 0.69$; $p = 0.003$). These findings support the concept that measurement of HIV-1 RNA in the easily accessible PBMC compartment is relevant for evaluating the potency of current and future antiretroviral therapies.

COMBINATION ANTIRETROVIRAL DRUG THERAPY can suppress HIV-1 replication,¹ resulting in marked clinical benefit.^{2–4} Plasma HIV-1 RNA quantitation is most widely used to assess an individual's virological status both for prediction of the subsequent clinical course⁵ and for monitoring the treatment response.⁶ Similar to plasma viral load, levels of cell-associated HIV-1 RNA are predictive of clinical progression.^{7,8} In addition, levels of HIV-1 RNA in lymphoid tissue, the presumed major reservoir of HIV-1,⁹ may provide information about residual viral activity in this reservoir for patients with undetectable plasma RNA levels.^{10,11} However, the limited accessibility of lymphoid tissue precludes the use of routine sampling for monitoring HIV therapy.

In this report we have determined HIV-1 RNA levels in plasma, PBMC, and lymphoid tissue in patients on potent long-

term antiretroviral therapy to assess the sensitivity of these virological measurements for detection of residual HIV-RNA.

HIV-1-infected, asymptomatic, treatment-naïve patients with >400 CD4 cells/ μ l at enrollment were randomly assigned to receive triple therapy [AZT 300 mg bid, 3TC 150 mg bid, and zidovudine (RTV) 600 mg bid] in a prospective, open-label trial called the Swiss EARTH study.¹² A subset of 10 individuals treated initially with triple drug regimens [50% female, average age 32 ± 5 years (mean \pm SD)] with follow-up data available at least up to Week 96 and plasma viral load suppressed permanently to levels below 50 copies/ml during the second and the third year of therapy was selected for the present study. Due to side effects, two patients (patients 9 and 10) discontinued RTV and in two patients RTV was replaced by zalcitabine (NFV) 750 mg tid (Table 1). From a subset of five patients bi-

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TABLE 1. PATIENT CHARACTERISTICS

Patient ID	Therapy start	Change (week ^a)	Plasma ^b		PBMC ^d		Lymphoid tissue ^d		Follow-up weeks
			Baseline	Nadir ^c	Baseline	Nadir ^c	Baseline	Nadir ^c	
1	AZT/3TC/RTV	AZT/3TC/NFV (7)	5028	≤5	99	5	nd	nd	96
2	AZT/3TC/RTV		1406	≤12	214	16	54056	6	114
3	AZT/3TC/RTV	AZT/3TC/NFV (12)	9995	≤9	252	≤5	115812	343	124
4	AZT/3TC/RTV		3725	<6	288	13	nd ^f	nd	96
5	AZT/3TC/RTV		32595	<7	431	6	557124	<12	132
6	AZT/3TC/RTV		30202	17	978	24	21025	6	108
7	AZT/3TC/RTV	AZT/3TC (80)	36835	≤10	2789	49	36450	11	126
8	AZT/3TC/RTV		9154	≤9	3155	111	192514	nd	126
9	AZT/3TC/RTV		35574	≤14	2138	49	nd	nd	102
10	AZT/3TC/RTV	AZT/3TC (13)	8555	≤8	244	≤4	14109	477	132

^aStudy week in which therapy was changed.
^bHIV-1 RNA copies/ml.
^cMean HIV RNA levels after study Week 47 (<signifies that undetectable values were obtained throughout the observation period and ≤ means that some HIV-RNA levels were detectable and some were not).
^dHIV-1 RNA copies/μg total RNA.
^eHIV-RNA levels at study Week 48 (<signifies the detection limit of an undetectable value).
^fnd, no data available.

lateral tonsil biopsies were obtained at Weeks 0, 4, 24, and 48 and from 1 subject at Weeks 0, 4, 24, 48, and 96. In one patient fine-needle biopsies from lymph nodes were available at Weeks 0 and 24.

After initiation of treatment, plasma HIV-RNA in all study patients reached levels below 50 copies/ml in a median (min; max) time of 12 (4; 36) weeks and subsequently remained below 50 copies for a median duration of 105 (60; 128) weeks. Overall, in 25% of plasma specimens obtained during the second and third year of therapy HIV-1 RNA (time points after Week 47) was measured at very low but detectable levels. For individual patients detectable plasma viral loads were observed with a frequency of 9% to 50% at time points after Week 47. From one patient (patient 6), all specimens and from two patients (patients 4 and 5) none were found to have detectable plasma HIV-1 RNA.

In contrast to plasma, HIV-1 RNA remained detectable in the vast majority (91%) of PBMC specimens. In seven patients (patients 1, 2, 4, 5, 7, 8, and 9) PBMC-associated HIV-1 RNA was detectable in 100% of the specimens. Lower frequencies (92, 89, and 42%, respectively) were observed in three patients (patients 3, 6, and 10, respectively). The mean PBMC-associated HIV-1 RNA levels measured after 1 year of therapy (>Week 47), henceforth referred to as nadir HIV-1 RNA levels, plateaued during the second and third year of therapy (Fig. 1B) as confirmed by the finding that no patient showed a significant trend for a decrease of PBMC-associated HIV-1 RNA during the second or third year of therapy (Spearman nonparametrical analysis, $p > 0.18$).

Similarly as in PBMC, very few specimens from lymphoid tissue had undetectable HIV-1 RNA: Only 3 of 27 samples were HIV-1 RNA negative: two from patient 5 and one from patient 8. The very low yield of total RNA extracted from each of these specimens may explain the negative results.

After initiation of therapy, changes in HIV-1 RNA levels in PBMC and lymphoid tissue paralleled changes in plasma HIV-

1 RNA (Fig. 1). HIV-1 RNA levels in plasma and PBMC were significantly correlated at baseline ($r^2 = 0.41$; $p = 0.05$; $n = 10$) and throughout the observation period ($r^2 = 0.46$; $p < 0.0001$; $n = 62$) (Fig. 2). Significant correlation of HIV-RNA levels in lymphoid tissue compared to either PBMC or plasma HIV-1 RNA levels was demonstrated for the whole observation period ($r^2 = 0.40$; $p = 0.001$; $n = 23$ and $r^2 = 0.54$; $p = 0.002$; $n = 15$, respectively) but not for the data at baseline ($p > 0.25$; $n = 6$).

Virological response¹³ to therapy was calculated as the ratio of the nadir HIV-1 RNA levels divided by the baseline level for each individual patient in plasma, PBMC, and lymphoid tissue. Virological responses in PBMC and plasma were highly correlated ($r^2 = 0.69$; $p = 0.003$; $n = 10$) (Fig. 3). This correlation remained significant, when the analysis was recalculated assuming that PCR-negative specimens contained zero copies ($r^2 = 0.54$; $p = 0.04$; $n = 8$). However, the comparison of virological response in lymphoid tissue to PBMC failed to show significant correlation ($p > 0.4$; $n = 6$).

Two main observations were made in this study:

1. In patients treated with potent antiretroviral therapy, PBMC-associated HIV-1 RNA persisted for up to 2 years or longer at levels as low as 5–10 copies/μg total RNA and did not show a tendency to decay further during the second year of therapy.
2. Virological response to therapy in plasma was mirrored by the response in PBMC-associated HIV-1 RNA with the measurement in the cellular compartment being much more sensitive.

Unlike plasma viremia, levels of HIV-1 RNA in PBMC and lymphoid tissue rarely became undetectable. PBMC-associated HIV-1 RNA was found to persist for up to 2.5 years of therapy. Furtado and colleagues¹⁴ recently reported persistence of PBMC-associated HIV-RNA in five patients under potent an-

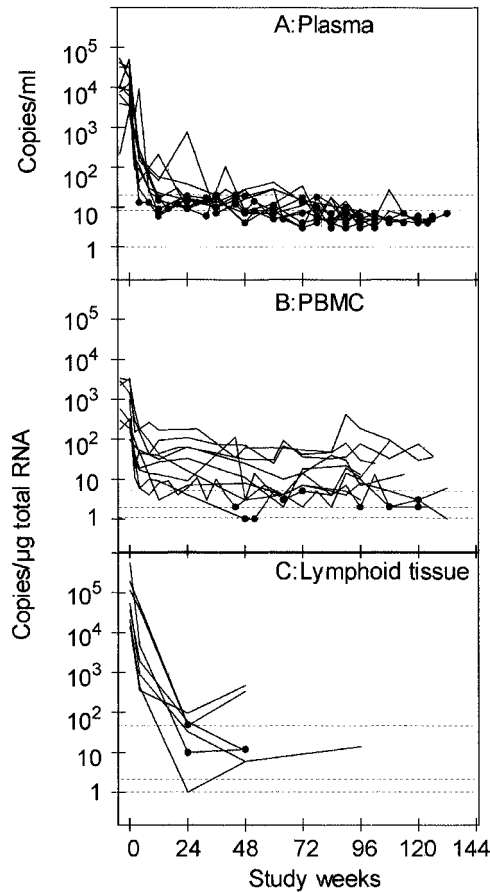


FIG. 1. HIV-1 RNA levels in plasma, PBMC, and lymphoid tissue during long-term antiretroviral therapy. Solid lines connect datapoints from individual patients. Filled circles indicate undetectable measurements and their individual limits of detection. Dotted lines parallel to x-axes show median, maximal, and minimal detection limits for RNA measurements in each compartment. (A) Plasma HIV-1 RNA was determined by the Amplicor HIV-1 Monitor Test and ultrasensitive modifications.^{34,35} (B) PBMC HIV-1 RNA. (C) HIV-1 RNA in lymphoid tissue. Lymphoid tissue biopsies were processed as described previously.¹⁷ Measurements of HIV-1 RNA in PBMC and lymphoid tissue were performed by a modification of the Amplicor Test.^{12,35}

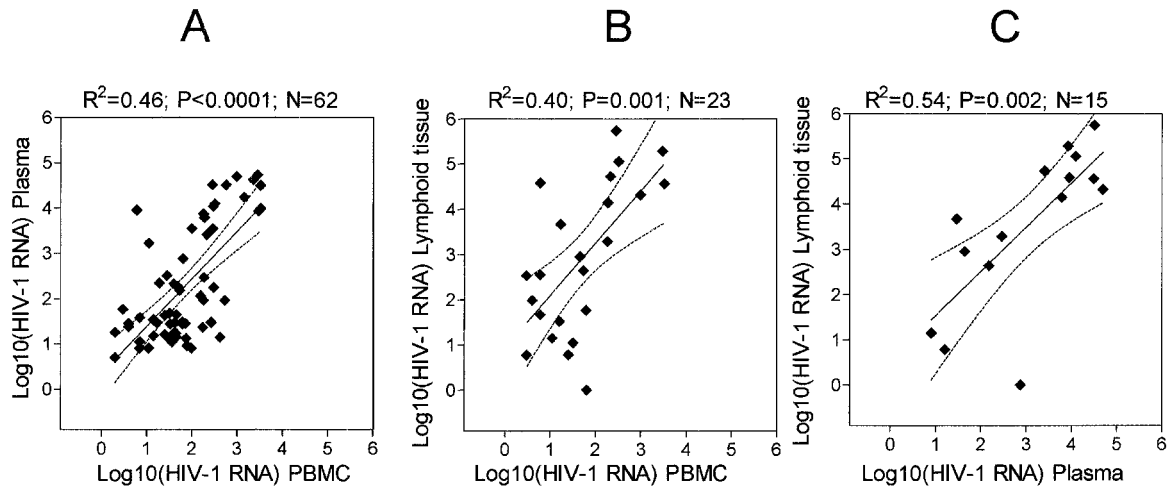


FIG. 2. Correlation between HIV-1 RNA levels in PBMC, plasma, and lymphoid tissue. Combined data from all patients during the whole observation period are shown. Detectable \log_{10} transformed HIV-1 HIV RNA measurements were used for this analysis. N , number of datapoints; R^2 , Pearson correlation coefficient; P , level of significance. Solid and dotted lines show calculated linear regression curves and their 95% confidence intervals, respectively, with the following underlying equations: (A) HIV-RNA in PBMC compared to plasma; $y = 1.05x + 0.34$. (B) HIV-RNA in PBMC compared to lymphoid tissue; $y = 1.15x + 0.93$. (C) HIV-RNA in plasma compared to lymphoid tissue; $y = 0.97x + 0.56$.

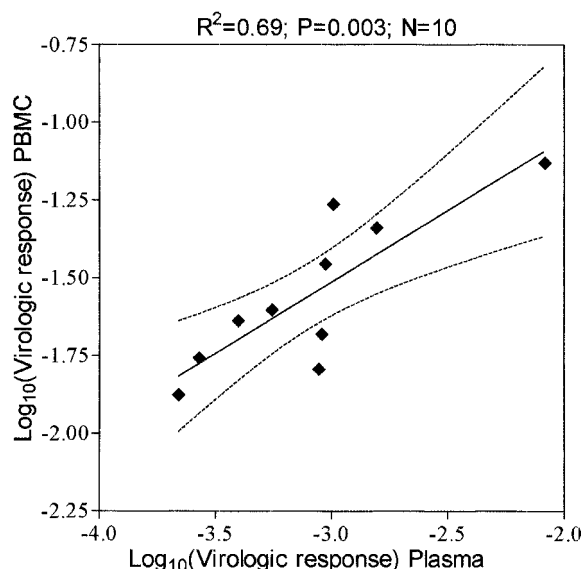


FIG. 3. Correlation between virologic response of HIV-1 RNA levels in PBMC and plasma to antiretroviral therapy. Virological responses¹³ of HIV-1 RNA levels were plotted as \log_{10} (nadir HIV-1 RNA levels/baseline HIV-1 RNA levels). *N*, number of data points; *R*², Pearson correlation coefficient; *P*, level of significance. The solid line shows the calculated linear regression curve and its 95% confidence intervals (dotted lines) with the following underlying equation: $y = 0.46x - 0.13$.

tiretroviral therapy. Our findings extend this observation showing that at least for the chronically infected asymptomatic patients studied here, such persistence can be stable in that no further decrease of PBMC-associated HIV-1 RNA was observed in the second year of treatment.

The question has been posed whether the residual cellular HIV-RNA persisting in PBMC reflects intracellular transcripts of infected cells or residual virions attached to the outside of uninfected cells¹⁵—in analogy to extracellular viral particles trapped in lymphoid tissue.^{16–18} Consistent with the hypothesis that residual PBMC-associated HIV-1 RNA may be, in part or entirely, extracellular, it was observed that 25% of plasma specimens tested after Week 47 showed detectable HIV-1 RNA at low levels (all below 50 copies/ml). This observation, in agreement with a recent report,¹⁹ shows that current antiretroviral therapies may not completely suppress virion production.

However, we did not observe elevated nadir levels of PBMC-associated HIV-1 RNA in the patients with actually detectable plasma viremia after 1 year of therapy (data not shown), which might be expected under the assumption that PBMC-associated HIV-1 RNA would merely reflect residual virions bound to the surface of uninfected cells.

If residual PBMC-associated HIV-1 RNA were located intracellularly, the question would arise whether such cellular transcripts originate from chronically or productively infected cells. Observations of other investigators indicate that both may

apply. In particular, there is evidence that active viral replication may persist in the presence of treatment with potent antiretroviral therapy.^{20–22} Conversely, the persistence of PBMC-associated HIV-1 RNA may be based on latency of long-lived infected cells^{23–25} transcribing HIV-RNA at very low levels. Reminiscent of our observations, uninduced J1.1 cells—chronically infected T-lymphocytes—have been reported to express low levels of predominantly unspliced HIV-1 RNA in at least a fraction of cells.²⁶

Comparison of HIV-1 RNA levels from the different compartments revealed that virological response in plasma mirrored response in PBMC with remarkable accuracy. The rationale for having defined virological response in our patients by the ratio of baseline and nadir HIV-1 RNA levels¹³ was based on observations that found “high baseline”^{27–29} and “high nadir levels”^{30,31} of plasma viremia to be predictors for virological failure of antiretroviral therapy. Reminiscent of the correlation of virological responses, PBMC HIV-1 RNA levels were also directly correlated with viral RNA levels in plasma at baseline and throughout the whole observation period. The finding that correlation was weaker ($r^2 = 0.46$) in the direct comparison than in the paired analysis of virological responses ($r^2 = 0.69$) may be due to the high patient-to-patient variation observed in PBMC-associated HIV-1 RNA levels (coefficients of variations at baseline and nadir of 111 and 120%, respectively). Correlation of PBMC-associated HIV-1 RNA levels with viral RNA load in lymphoid tissue was also demonstrated. However, baseline HIV-1 RNA levels as well as the virological response in lymphoid tissue failed to show significant correlation with either plasma or PBMC. Such discordance can be expected because the vast majority of HIV-1 RNA in lymphoid tissue of untreated patients represent extracellular virions bound to follicular dendritic cells,¹⁶ which may be saturated at baseline.^{16,32} It has also been claimed that HIV-1 RNA associated with mononuclear cells in lymph nodes reflects plasma viremia better than follicular dendritic cell-associated HIV-1 RNA.³² Confirming the latter assumption, strong correlation between viral load in purified lymph node mononuclear cells and PBMC viral load has been reported.³³

In conclusion, the data presented here suggest that measurements of PBMC-associated HIV-1 RNA may permit assessment of virological response even among patients with permanently undetectable plasma viral loads, when the distinction between “potent” and “very potent” antiretroviral therapy may otherwise be impossible.

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